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Studies on Leaf Burn of Pear Trees

VIII. Stomatal Responses to Water and to Light of the Leaves of Pear Trees in Summer

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Introduction

It was shown in previous papers[5)6)] that a dull closing action of the stomata was a main factor in developing leaf burn in pear trees. However, the dull closing action of the stomata should be investigated in detail in relation to when it develops, how it develops, what it plays in the feedback systems of stomatal action[4)] and whether the dull opening action exsists or not. Although continuing investigations will show the nature of these dull stomatal closing action, the present paper, in the meantime, is a record of the observations of stomatal responces to water and light of the leaves of the several cultivars of pear trees in summer. Further, the present paper describes the differences in transpiration resistances resulting from these differences in the stomatal responses and reports the effects of several antitranspirants on both the leaf resistances and leaf burn.

Materials and Methods

1. Measurement of stomatal responses of detached leaves to water.

Samples used were taken from 3 cultivars of 7-year old pear grown in the orchard of the Faculty of Agriculture, Yamagata University : 'Bartlett' (most susceptible to leaf burn), 'Grand Champion' (susceptible) and 'Red Bartlett' (resistant). The detached leaves from the base of the selected shoots were immersed in water for 30 minutes and then illuminated with 4 incandescent lamps placed 30 cm away (5,000 lx.) at a constant temperature and VPD (25°C and 1.2 mmHg) in a chamber for 3 hours. Then the leaves were hung before the conversion of VPD to 5.4 mmHg in the chamber. The measurement of stomatal resistance of each sample with a diffusive porometer of VAN BAVEL's type (Rate Hygrometer, Ennis and Association) was followed by weighing at 5 minutes interval for 30 minutes. Finally, the leaves were saturated with water for 4 hours with polyurethane foam soaked water, then dried at 105°C for 24 hours and reweighed. Water saturated deficits (W. S. D.) were calculated. These measurements were repeated 5 times per cultivar.

2. *Measurements of stomatal responses of the leaves on the cut shoots supplied with water to light.*

The shoots of the cultivars mentioned above were cut in a beaker full up water and examined for stomatal opening and closing in a chamber the following controlled conditions : an initial light period of 3 hours at 25°C in temperature and 40∼70% R. H. ; a dark period of 80 minutes at 0 lx., 22°C in temperature and 70∼85% R. H. ; a second light period of 20 minutes at 25°C in temperature and 40∼70% R. H.. The irradiance in the chamber was supplied by 4 metal halide vapor and color-improved mercury lamps. In the light period, the illumination was about 30 klx. on horizontal plane at the top of the shoots but it was about 10 klx. on the inclined leaves attached at lower positions of the shoots.

3. *Ventilations of attached leaves of pear trees.*

Another set of stomatal responses to water were investigated using intact leaves. Acceleration of leaf transpiration was induced by ventilations. Two electric motor fans were placed 30 cm away from the leaves at the base in the shoots of both 'Bartlett' and 'Red Bartlett', which gave a wind speed of about 2 m sec^{-1}. The transpiration resistances of the leaves were measured at 10 minutes interval for 90 minutes in the second scattering daylight.

4. *Treatments of conversion of soil water tension in pots.*

A pair of 6-year old potted pear trees of 2 cultivars, 'Bartlett' and 'Flemish Beauty' (resistant), were treated with a conversion of soil water tension from 0 to 600 mmHg and with another rapid conversion from 600 to 0 mmHg. The changes in diameters of 3 fruits per cultivar were measured in order to estimate the changes in water status in the trees using a "electro-contact calliper", designed

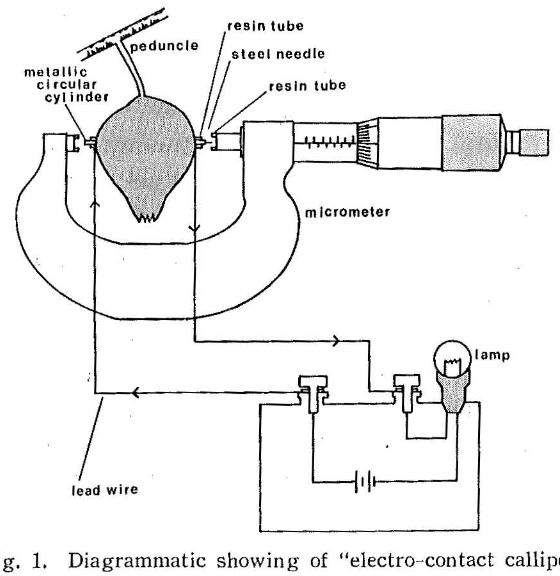

Fig. 1. Diagrammatic showing of "electro-contact calliper".

by the authors and illustrated in Fig. 1. In the equipment, when contact is made, electricity flows through the metal cylinders, spindle and anbil, the body of the micrometer, lead wires and a lamp without contact pressure. Soil water tensions were measured by tensiometers buried in the pots.

5. *Measurements of stomatal responses in the field.*

From the bases of the shoots and the spurs of 3 trees in each of 7-year old pear cultivars, ('Bartlett', 'Grand Champion' and 'Red Bartlett'), 3 leaves in the second scattering daylight were selected at each measurement time. Their transpiration resistances were measured at 6 ~ 7 A. M., 12 A. M. ~ 1 P. M. and 9 ~ 10 P. M., respectively, on every day without rainfall and dew formation from late June to early September.

6. *Sprays of antitranspirants.*

Each of 5 antitranspirants were sprayed at 3 secondary scaffold branches within 'Bartlett' pear trees mentioned above in late July and in middle August. Transpiration resistances of the leaves were measured in the second scattering daylight at 3 P. M. each day for a week or more from the day following the spraying. Burned leaves were counted each day at 5 P. M., and the inhibition of development of leaf burn were valued in percent on the base of the total leaves on the sprayed branches.

7. *The relation between transpiration resistances and stomatal apertures.*

Samples were collected from the spurs of 'Bartlett' pear trees in early July. The measurement of transpiration resistance was followed by measurements of stomatal aperture with both direct observation by a light microscope (NeoPark, Olympus Co.) and infiltration of kerosene, on each detached leaf which was desiccated slowly in light.

8. *Meteorological elements.*

Meteorological elements were calculated from the recording data of the Meteorological Observatory of Faculty of Agriculture located at about 50 meters from the orchard and of the Meteorological Observatory at Takasaka Agricultural Farm located at about 2 km away.

Results

1. *Stomatal responses of the detached leaves to water.*

The comparison of the responses of the cultivars is shown in Fig. 2. As the W. S. D. rose, so did the resistance on each cultivar. In particular, that of the leaves of the base of the shoots and of the spurs of 'Red Bartlett' rose rapidly at about 10 minutes from the beginning of the treament, when their W. S. D. reached at about 30%. In contrast, that of 'Bartlett' and 'Grand Champion' rose slowly and reached double their initial values finally only when their leaves were desiccated severely about 60% in W. S. D..

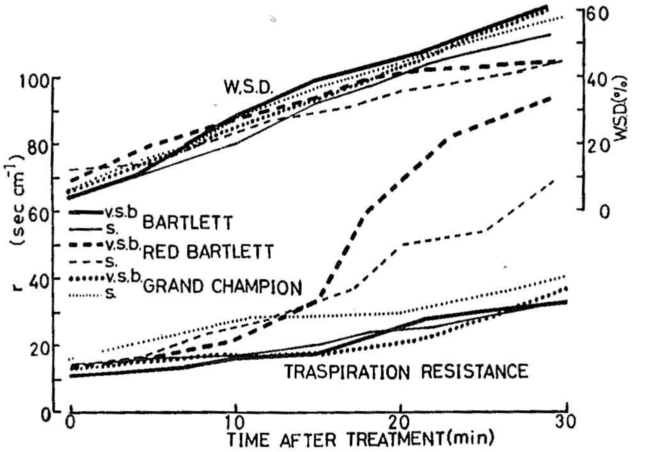

Fig. 2. Relations between transpiration resistances and W. S. D. of detached leaves of 'Bartlett' desiccated in 25°C, 5.4 mmHg of VPD and 5,000 lx.. V. s. b. and s. indicate the leaves of the base of the shoots and spur leaves, respectively.

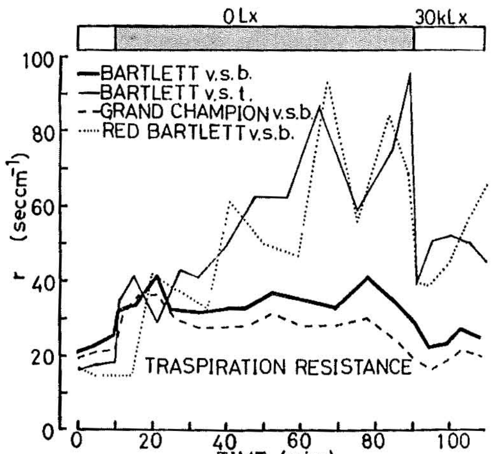

Fig. 3. Relations between transpiration resistances and light conversions of the leaves on the cut shoots at 25°C. V. s. t. indicates the leaves of the terminal of the shoots.

2. Stomatal responses of the attached leaves on the cut shoots to light.

The comparison of the responses of the cultivars is shown in Fig. 3. After about 3 hours of the pretreatment in light, the first measurements of the resistances indicated that the resistances of the leaves of the base of 'Red Bartlett shoots and of the leaves of the terminal of the shoots of 'Bartlett' were lower than that of the base of both 'Bartlett' and 'Grand Champion'. After conversion from light to dark, formers two rose rapidly, but latters two rose slightly and maintained more constant and lower values than the formers. As relighted, the

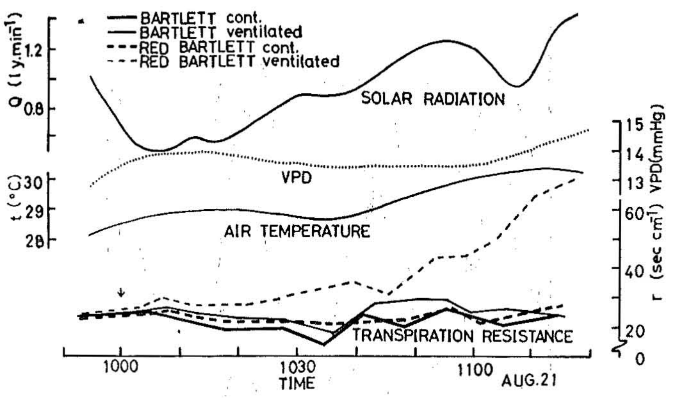

Fig. 4. The effects of ventilation on transpiration resistances of the leaves at the base of the shoots of 2 cultivars of pear trees in the field.

formers did not return to the initial values for 20 minutes, but the latters did quickly.

3. *The effects of the ventilation on the transpiration resistances of attached leaves of pear trees.*

From the beginning of the treatment, as indicated in Fig. 4, the resistances of the ventilated leaves of 'Red Bartlett' rose gradually and reached threefold value in 90 minutes. Differently, that of the ventilated leaves of 'Bartlett' remained constant at the same levels as the contrast when the natural wind velocities were below 0.5 m sec^{-1}.

4. *The effects of rapid changes in soil water tension in the pot on the stomatal resistances.*

The results are indicated in Fig. 5. In the experiment, as shown in the upper part of the figure, high temperatures and severe dryness existed in the glasshouse. When soil water tension fell rapidly and fruit diameter recovered as indicated in the middle part of the figure, the resistances were relatively high, especially in the leaves of the terminal of the shoots of 'Bartlett'. In the drying treatment of wet soil, the resistances of the leaves of the base of the shoots of 'Bartlett' were lower than the others on Jul. 23 when contraction of the fruits began.

5. *The comparisons among cultivars and kinds of the shoots of stomatal responses to water and to light of the leaves in the field.*

The total number of the measurements were 28 in the morning, 48 at noon and 28 at night. Among these, the results on continuous, fine and dry days are shown in Fig. 6. In 1976, rainy year, soil water was abundant, though perhaps relatively low temperatures and dry air conditions made these resistances generally high. 'Bartlett', most susceptible to leaf burn, had relatively low resistances ; nevertheless it showed complicated changes in resistances owing to the changeable weathers.

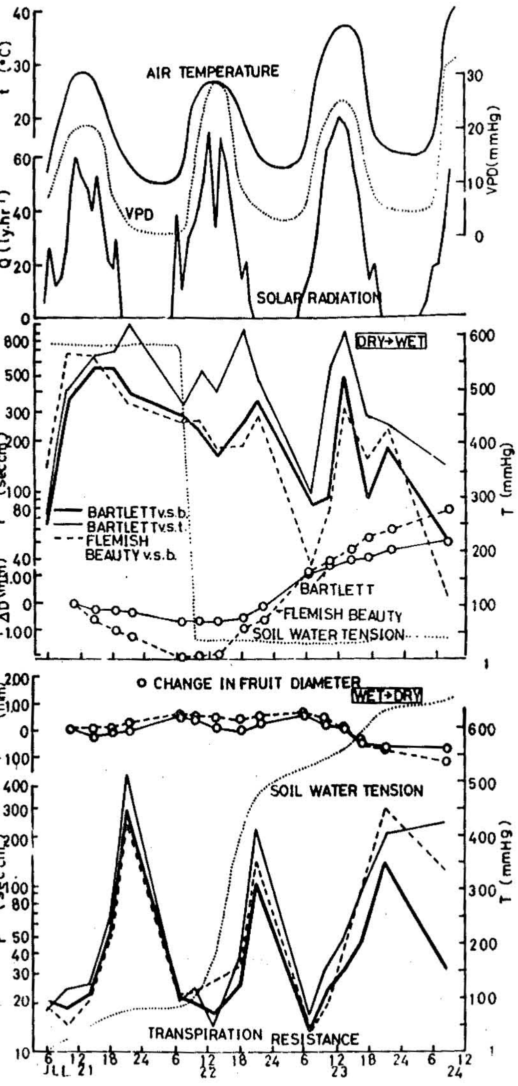

Fig. 5. Changes in the transpiration resistances of the leaves of 2 cultivars of pear potted in a glasshouse caused by changes in soil water tension.

After conversion of all data from resistances to conductances, the mean conductance of each cultivar and of each kind of shoots was compared, as indicated in Table 1. All over cultivars, the mean conductances of the leaves of the base of the shoots were higher that of spur leaves. Both in the morning and at noon, the conductances of the leaves of the base of the shoots of 'Grand Champion' and of 'Bartlett' differed from that of 'Red Bartlett' with 5 % level by DUNCANS multiple range test. But, mean separations among the others were impossible by the test perhaps owing to the changeable weather conditions. At night, similar

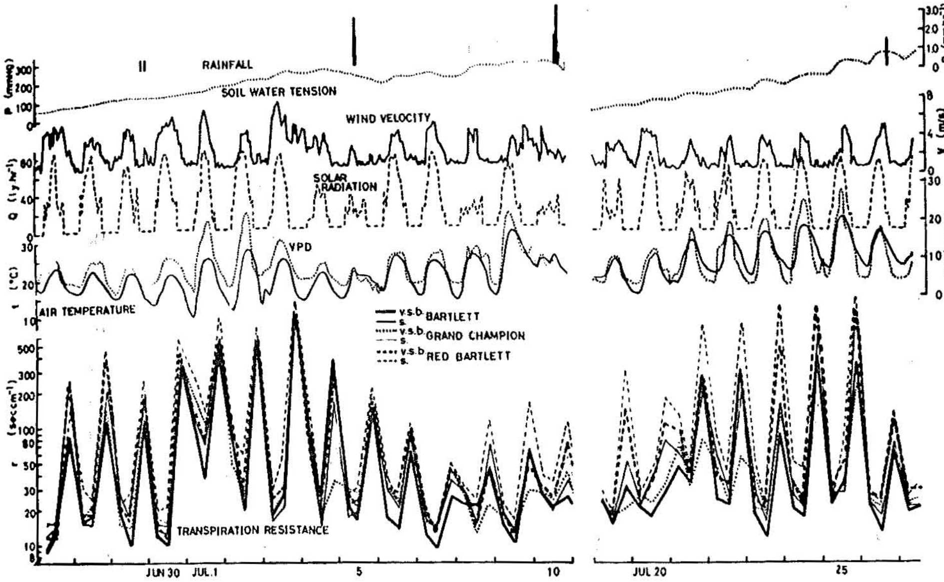

Fig. 6. Daily changes in transpiration resistances of 3 cultivars of pear trees measured at 6 A. M., 12 A. M. and 9 P.M..

Table 1. Mean leaf conductances of 3 cultivars of pear trees. V. s. b., v. s. t. and s. indicate leaves of the base of the shoots, of the terminal of the one and spur leaves, respectively.

Cultivars and kinds of the shoots	Times of the measurements 6-7 A. M. n=28	12 A. M.-1 P. M. n=48	9-10 P. M. n=28	Selected days 12 A. M.-1 P. M. n=16
	cm sec^{-1}	cm sec^{-1}	cm sec^{-1}	cm sec^{-1}
'Bartlett' v. s. b.	0.0628(100)a[z]	0.0802(100)a	0.0135(100)ab	0.0669(100)Aa
'Bartlett' v. s. t.	0.0488(78)ab	0.0652(81)ab	0.0078(58)bc	0.0542(81)Bb
'Bartlett' s.	0.0497(79)ab	0.0613(76)ab	0.0104(77)ab	0.0533(80)Bbc
'Grand Champion' v. s. b.	0.0494(79)ab	0.0638(80)ab	0.0175(130)Aa	0.0507(76)Bbc
'Grand Champion' s.	0.0433(69)b	0.0567(71)b	0.0120(89)ab	0.0441(66)BCcd
'Red Bartlett' v. s. b.	0.0511(81)ab	0.0616(77)ab	0.0102(76)ab	0.0478(71)BCbc
'Red Bartlett' s.	0.0424(68)b	0.0573(71)b	0.0050(37)Bc	0.0361(54)Cd

[z]Mean separation by DUNCANs multiple range test, 1 % level (big letters), 5 % level (small letters)

phenomena were noted ; but, the stomata on the leaves of the terminal of the shoot of 'Bartlett' closed tightly, and the conductances of the leaves of 'Grand Champion' were relatively high. Next, 16 days on which the weather changed from wet to dry were picked from among all data of the meteorological elements. Then the mean conductances at noon on these days were compared as shown in the last column on the right in Table 1. According by DUNCANS test, the mean separations of the conductances were possible with 1 % level between the leaves

Table 2. The effects of the sprays of 5 antitranspirants on the transpiration resistances and on the developments of leaf burn of the leaves in the second scattering daylight of 'Bartlett' pear trees.

	Mean transpiration resistance (sec cm^{-1})		Percent of burned area in the total leaf area	
Antitranspirants	First spray (Jul. 27-Aug. 6)	Second spray (Aug. 18-24)	First spray	Second spray
ABA 10 ppm*	36.7 (175)	36.0 (120)	0.43	0.11
SHIONOX 3.3%	30.0 (143)	32.0 (114)	0.42	0.19
CARPLEX 3.3%	24.2 (115)	28.1 (100)	1.20	0.14
CLEFNON 1.2%	26.8 (138)	31.2 (102)	0.33	0.01
OEDGreen 2.5%	28.9 (138)	31.2 (111)	0.51	0.16
Water (cont.)	21.0 (100)	28.0 (100)	1.41	0.22

*ABA treatment contains 0.02 percent, of a surfactant, Tween-20.

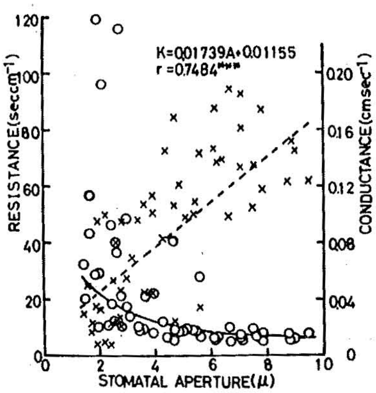

Fig. 7. Relations between transpiration resistances, leaf conductances and stomatal apertures determined by direct observation method of the leaves of the base of shoots of 'Bartlett' pear in early July.

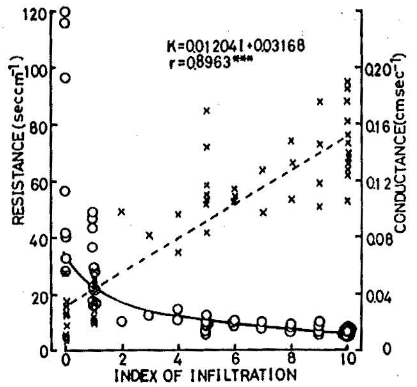

Fig. 8. Relations between transpiration resistances, leaf conductances and stomatal apertures using the infiltration method of the leaves of the base of the shoots of 'Bartlett' pear in early July.

of the base of the shoots of 'Bartlett' and others, with 5 % level between the spur leaves of 'Bartlett' and 'Red Bartlett'. The mean resistances, reconverted from the mean conductances, of the leaves of the base of the shoot of 'Red Bartlett' were 1.5 times as high as that of 'Bartlett'.

6. The effects of the several antitranspirants on both the resistances and leaf burn of 'Bartlett' pear trees.

The total percent of leaf burn and the mean resistances for the periods are indicated in Table 2. From the time of the first spray on Jul. 27 to Aug. 6, clear days continued, but then a heavy rain of about 100 mm washed away these antitranspirants from the leaves. During the second spray experiment, Aug. 18-24, when wet weather dominated, results on both leaf burn and the resistances were obscure. As shown in Table 2, in the first spray, ABA, SHIONOX and OEDGreen had 175, 143 and 138 of the relative resistances to contrast (=100), respectively. The inhibition of leaf burn was the least in CARPLEX.

7. The relation between transpiration resistances and stomatal apertures.

The relation between the resistance measured by the diffusive porometer of this type and the stomatal apertures measured by both the direct observation and the infiltration series, is indicated in Fig. 7 and 8. The index of infiltration is given by combining the area infiltrated with kerosene and time required. The wider stomatal pores, the greater the index. From two hyperbolas, it appeaed that apertures less than about 2 micron with direct observation method and less than about 1 of index of infiltration cannot be measured. The correlation between the conductances converted from resistances and the apertures by the infiltration method were higher than that by the direct observation method.

Discussion

In a recent review of the model of stomatal action, RASCKE (1975) stated that stomatal responses to light is attributed to the responses to CO_2 and that stomatal responses to water contain two feedbacks of 'hydropassive' and 'hydroactive'[4).

In the present study, the stomatal responses to water will contain the two feedback systems above, but these cannot be studied separately. Responses to CO_2 should be measured : however many stomatal physiologists have studied such responses using detached leaves (especially epidermal strips), while in relation to leaf burn, it is important to investigate stomatal action using intact leaves.

Several approaches were employed to induce internal water deficits of leaves in the present study. In the treatment of the desiccation of detached leaves, the leaves dried up in short time owing to absense of water supply and to weak water tension within leaf veins. But, surprisingly, in those leaves susceptible to leaf burn dried to 50% in W. S. D., dull closing of the stomata were still seen as indicated in Fig. 2, even though hydroactive stomatal closing needs several minutes or more after the time when water loss of the critical range occurs[4).

As the transpiration resistances of the leaves of 'Bartlett' were lower than that of 'Red Bartlett', which were ventilated, it appears that the leaves of 'Bartlett' have been dehydrated more severely than that of 'Red Bartlett' by the difference in transpiration rate (assuming that water supply from the petiole and environmental factors were equal). Nevertheless, the resistances of ventilated leaves of 'Bartlett' had same level as those of contrast. This was not seen in 'Red Bartlett' and can be taken as evidence of dull stomatal closing against water deficits.

As soil water tension in the pot rises, water supply from the soil will decrease. In this case, the linkage of the stomata to plant hydraulics will be important. In the present study, rapid changes in soil water from wet to dry caused fruit contractions. However, when leaf burn develops in the field, such a rapid decrease in soil water and fruit contraction in appearances seldom happens. When soil water changed from dry to wet, and when fruits began to expand, stomata did not open directly. It appears to be an 'after-effect', especially in young leaves. The 'after-effect' can be said to be the effect of accumulation of ABA[3]. Stomatal responses to light of the attached leaves of cut shoots suppling water differs greatly among the cultivars. In the field experiment, the stomatal responses to environmental factors were more complicated. It is said that stomatal responses to water have a 'threshold'[3)5)6)]. All measured days included several days of cloudy and humid weathers when wet soils exsisted ; on such days, the water status of the leaves might not reach at the 'threshold'. Therefore, the variance of the resistances and the conductances were large in total, and mean separations were impossible by DUNCANS test. Thus, only the data from the days in which weather changed from wet to dry give significant mean differences, as indicated in Table 1. Because the resistances have different variances with the mean values[5], therefore resistances should be converted to logarithm form or reciprocal form in analysis of variance, in the latter case, to conductances[5]. The differences in the resistances in the morning and at noon will be the difference in stomatal responses to water among cultivars. Similarly, the differences between day and night will be the differences in stomatal responses to light. Such differences between the resistances of the leaves of 'Bartlett' and 'Red Bartlett' will be usefull in determing a ratio of the inhibition of transpiration for an antitranspirant which prevents development leaf burn but inhibits photosynthesis as little as possible. ABA was the most effective in relation to the ratio. However with the spray of ABA, leaf burn could not be prevented completely. Generaly, cheaper antitranspirants will be used. These problems will be resolved with more detailed investigations in the area of spray of antitranspirants and of leaf water relationships.

When leaf burn develops, a lower resistance of a leaf susceptible to leaf burn will induce a lower leaf water potential. Conversely, a continuous status with such a low leaf water potential will push up gradually the leaf resistance to a critical range of a higher one as discribed in Fig. 6. As indicated in Fig. 7 and 8, and as mentioned by BURROW et al. (1976), for ranges of the resistances below 1 micron of stomatal aperture, it will not be possible to use the older methods either of these dull closing of stomata or antitranspirants. Rather, diffusive porometer must be used. By using the porometer, the nature of the dull closing of stomata will be able to be explored thoroughly. It appears that the authors were the first to use this instrument to study on leaf burn[6] ; in that study, a GRIVE's type porometer was used which was more inconvenient than the type employed in the present study.

Summary

Several investigations have been made on the stomatal responses to water and light, especially on the dull closing action, in the leaves of pear trees which differ in the susceptibility to leaf burn. Stomatal movements were measured with a type of VAN BAVEL diffusive porometer. The results may be summarized as follows.

Different stomatal responses to water were found by means of the treatments of desiccation of detached leaves, of the treatments of ventilation of attached leaves and of the treatments of soil water stress in the pots. The closing action of the stomata of the leaves of 'Bartlett' (highly susceptible to leaf burn) and 'Grand Champion' (susceptible) were dull, but one of 'Red Bartlett' (resistant) was clear.

Similarly, different stomatal responses to light of the leaves among the cultivars were found. The closing action of the stomata of 'Bartlett' and 'Grand Champion' in the dark period were dull while that of the latter was clear.

In addition, the stomatal responses to water and light were investigated using attached leaves of the 3 cultivars in the field in the morning, at noon and at night for a long period in summer. In spite of changeable weathers during this period, the differences in transpiration resistances were observed among the cultivars and the kinds of the shoots. Especially, for the results on the day when the weather changed from wet to dry, the differences of mean conductance, reciprocal of resistance, between 'Barteltt' and 'Red Bartlett' were separated by DUNCANS multiple range test with 1 % level.

Five antitranspirants were sprayed on 'Bartlett'. The relative degree of the inhibition of both transpiration resistances and development of leaf burn was investigated.

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西洋ナシの葉やけに関する研究（第8報）
夏季における西洋ナシの葉の気孔の水と光に対する反応

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要　　　約

葉やけの発現性のそれぞれ異なる品種の葉を供試し，水湿と光条件が気孔の開閉機能におよぼす影響を調査した．

葉面の蒸散抵抗の測定には，VAN BAVEL 型の蒸散抵抗計を用いた．

その結果の要約は次のようである．

乾燥処理（切断葉），送風処理（着生葉）ならびに鉢植え供試樹の鉢土壌の水分調節などを行い，水に対する気孔の反応をみたところ，いずれも，葉やけ発生の甚（バートレット），多（グランドチャンピオン）品種の気孔閉鎖に鈍化がみられた．しかし，葉やけの発生しにくいレッドバートレット，フレミッシュビューティでは，処理区においても気孔の閉鎖運動は鋭敏に行われた．

着生葉（切枝水ざし）を用い，気孔の光に対する反応をみたが，水に対すると同様に，葉やけ発生易品種の気孔の閉鎖は暗黒条件下でも鈍く，難品種レッドバートレットのそれは敏感であった．

ほ場自然条件下の着生葉につき，毎日，朝，昼，夜の3回，蒸散抵抗を測定し，これを長期間継続した．その結果，平均的蒸散抵抗についてみると，気象の複雑な変化があったにもかかわらず，品種間の蒸散抵抗（又はコンダクタンス）に差がみられた．とくに日気象が湿潤から乾燥にむかうようなタイプの日のみについて比較すると，葉やけを生じやすいバートレットと，レッドバートレットなど発生しにくい品種との間に，DUNCAN の多重検定により1％レベルで有意な差がみられた．

各種の蒸散抑制剤を散布し，蒸散抑制率ならびに葉やけ発生防止効果を調査したところ，両者に対する散布効果はみられたものの，葉やけを完全に予防するには至らなかった．